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The Efficacy of Autologous Nanofat Versus Enhanced Nanofat with Adipose-Derived Stem Cells Transfers in the Treatment of Atrophic Post Acne Scars

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Authors' contributions

This work was carried out in collaboration among all authors. Author ME designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HA and ME managed the analyses of the study. Author SG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Acne scarring is a visible reminder of acne vulgaris resulting from inappropriate healing of active lesions. Yet, no golden standard is present. Nanofat is an emulsified homogenous suspension of adipose tissue rich in adipose derived stem cells (ADSCs). Its application serves regenerative purposes.
Objective: To evaluate efficacy of nanofat versus enhanced nanofat with adipose-derived stem cells transfer in treating atrophic acne scars.
Methods and Materials: This study was carried on 40 patients with atrophic acne scars; grouped into two groups. Group A: 20 patients; treated with nanofat with platelet-rich plasma (PRP) and group B: 20 patients; treated with enhanced nanofat with ADSCs and PRP. Histopathological

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examination was done before and 6-months after treatment, specimens were stained by haematoxylin and eosin, Mallory trichrome and Verhoeff-Van Gieson.
Results: In both groups; 70% showed excellent and very good improvement and patients' satisfaction was 60% for both groups. Histopathological examination revealed increase in epidermal thickness, formation of new collagen and elastic fibers without significant difference between both groups.
Conclusion: Nanofat transfer is easy, cost-effective, and safe for treating acne scars, compared to enhanced nanofat with ADSCs transfer which is costly and time consuming

Keywords: Acne scars; nanofat; adipose-derived stem cells; platelet-rich plasma; stromal vascular fraction.

ABBREVIATIONS

ADSCs : Adipose derived stem cells
GFs : Growth factors
H & E : Haematoxylin and eosin
PBS : Phosphate buffer saline
PRP : Platelet rich plasma
SVF : Stromal vascular fraction

1. INTRODUCTION

Acne vulgaris is a common multifactorial skin disorder of the pilosebaceous unit. Despite the availability of different topical and systemic treatments, the development of scarring is a common outcome, with an estimated prevalence up to 11–14% among acne patients [1]. Two forms of scars are identified: hypertrophic and atrophic. Different modalities can be employed in managing acne scars; chemical peelings, physical methods (lasers, intense pulsed light, cryotherapy), surgical modalities (dermabrasion, punch excision, subscision) and fillers. Up till now, the treatment of acne scars remains challenging [2].

Adipose-derived stem cells (ADSCs) have been used for regenerative purposes because of their extensive proliferative capacity to differentiate into multilineages [3]. Growth factors (GFs), cytokines and molecules secreted by ADSCs are responsible for the anti-inflammatory, angiogenic, regenerative properties, remodeling and antiscarring process [4].

Stromal vascular fraction (SVF) of adipose tissue holds ADSCs together with a heterogeneous population of other cell types; preadipocytes, endothelial cells, pericytes, haematopoietic-lineage cells, and fibroblasts**.** SVF may be employed directly or cultured for selection and expansion of an adherent cell population, ADSCs [5]. There is asynergistic interaction between the different cell types in SVF which is reinforced by the angiogenic superiority of SVF when compared to the ADSCs [6].

However, the expensiveness of SVF enzymatic isolation and the time allotted to collagenase digestion render this difficult to provide in clinical routine. These barriers consequently necessitate to find more efficient technique to produce SVF cells. New hope was born in 2013 with the first description of mechanical digestion of adipose tissue, a process the authors imperfectly termed ''nanofat grafting'' [7].

In nanofat, normal fatty tissue structure is lost, adipocytes are destroyed and turned into emulsion containing a large number of good-quality stem cells demonstrated by isolation and culture. [7] consider nanofat as an in vivo tissue-engineering process. In fact, the major effect of nanofat is due to stem-cell activity. In nanofat, the SVF is not purified but is mixed with oil and dead adipocyte fraction. However, in [7] opinion, isolating SVF from the nanofat before injection would be complicated, time consuming, and expensive. Moreover, it is known that apoptotic cells attract macrophages that induce GFs and play important role in regeneration of the damaged tissue. For this reason, injection of fragmented adipocytes may play a role in stem-cell differentiation and tissue regeneration [7].

Platelet-rich plasma (PRP) positively promotes the induction of pluripotent stem cells in adipocytes [8].

The aim of this study was to evaluate the role of nanofat versus enhanced nanofat transfer in treating atrophic acne scars.

2. PATIENTS AND METHODS

This prospective randomized clinical trial included 40 patients with atrophic acne scars who had no isotretinoin or any resurfacing

techniques within the previous 6 months. The exclusion criteria included patients with hematological disorders, systemic or connective tissue diseases, pregnant females, patients with active acne lesions.

Patients were divided blindly by closed envelope method into 2 groups:

Group A; 20 patients were subjected to autologous nanofat transfer and Group B: 20 patients were subjected to ADSCs enriched nanofat transfer. PRP was added to both groups.

All patients were clinically evaluated, and demographic data were reported. They were subjected to full history taking, thorough general and cutaneous examination and thorough clinical evaluation of acne scars types and grading according to qualitative and quantitative grading of [9,10] (Table 1). The following investigations: complete blood count, bleeding time, prothrombin time, hepatitis B, hepatitis C and HIV tests, pregnancy test were done. Patients were advised to stop any medications that increase bleeding tendency as non-steroidal anti-inflammatory drugs. Systemic antibiotic had been started 48 hours before the procedure and continued for 1 week after the procedure.

Digital photographs were taken preoperatively, at 3- and 6-months follow-up period at the same distance, patient's position and illumination.

Lipoaspiration was done under local anesthesia at surgical theatre under complete aseptic precautions. Infiltration of epinephrine 1:1,000,000, and local anesthesia 1mg/kg in normal saline solution was performed in the lower abdomen. Harvesting fat was performed using 2 or 3 mm, three holes, blunt tip cannula attached to a 60 ml Luer-lock syringe [11]. The aspirated fat was collected in 50 ml tubes under complete aseptic conditions and was used either to prepare nanofat only or to isolate ADSCs and enrich the nanofat. Compression garment at site of harvesting was applied for 48 hours.

Nanofat preparation: Harvested fat was centrifuged at 3000 rpm for 3 min. Supernatant oil and fluid at the dependent portion of the syringe were removed. The lipoaspirate was mechanically emulsified by shifting the fat between two 10-cc syringes connected to each other. After 30 passes, the fat changed into an emulsion which was filtered over the sterile nylon cloth and this effluent is called "nanofat" (Fig. 1).

Adipose-derived stem cells preparation: Lipoaspirate was extensively washed in equal volume with phosphate buffer saline (PBS) for 3-4 times, 5 minutes each until the medium became clear. Washed adipose tissue (25ml) was mixed with PBS (10ml) to reach 35 ml to which 35 ul of collagenase (NB 4, Nordmark) was added. The mixture was placed in 50 ml conical tubes in water bath at 37□ for 1 hour. Vigorous manual shaking for 5-10 seconds every 15 minutes was performed till adipose tissue became soup-like. This digested adipose tissue was centrifuged at 2180 rpm for 10 minutes. The supernatant part was discarded leaving the infranatant part which is the pellet. The pellet was resuspended in 50 ml solution (44 ml DMEM, 5 ml 10% fetal bovine serum, 1 ml Penicillin-Streptomycin-Amphoteracin B mixture). Second centrifugation at 2180 rpm for 5-10 minutes was done and the infranatant was filtered through strainer (70 um) to remove unwanted debris. Third centrifugation of the filtered pellet at the same speed for 5 minutes, and the infranatant part represented the new pellet which was carried on PRP and enriched the prepared nanofat.

Platelet-rich plasma preparation: 10 cc of venous blood have been collected under complete aseptic conditions into a tube containing sodium citrate (10:1). The citrated blood was subjected to 2 centrifugation steps. The initial centrifugation at 1000 rpm for 10 minutes and second centrifugation at 2000 rpm for 10 minutes, typically, the lower 1-2 cc of the plasma, was yielded as PRP concentrate [12]. The PRP was then used to both groups. The prepared nanofat or enriched nanofat were injected intradermally through 27-gauge needle into acne scars till the appearance of yellowish bleb in injection site. Ice packs were applied on the injection sites for 15 minutes/hour for the first 4-6 hours.

During follow-up, the clinical assessment was done by 3 blinded observers according to qualitative and quantitative scores of Goodman and Baron [9,10]. In addition, the quartile grading scale was applied; poor improvement(<25%), good improvement (25-49%), very good improvement (50-74%), excellent improvement (≥75%) [13].The patient's opinion whether not satisfied, slightly satisfied, satisfied or very satisfied. Patients were informed to report any complications occurred.

Histopathological assessment of the scar was done through3-mm punch skin biopsies from the same lesional sites before and 6-months after

treatment. Specimens were stained with haemtoxylin and eosin (H&E), Mallory trichrome and Verhoeff-Van Gieson stains.

For the composition of the nanofat, a sample of it was examined by transmission electron microscopy.

3. RESULTS

The patients' demo graphic data were summarized in Table 2. There was no statistically significant difference between the 2 groups regarding age, sex and acne scar types. Based on the qualitative grading of acne scars; In group A: before treatment, 40% were grade 4, 40% were grade 3 and 20% were grade 2. After 6-months follow-up; 10% were grade 3, 50% were grade 2, and 40% were grade 1. There was a significant difference in grading between before, after 3-months and 6-months follow-up (P=0.010*, P=0.001* respectively). In group B, before treatment, 60% were grade 4, 30% were grade 3 and 10% were grade 2. After 6-months follow-up; 40% were grade 3, 30% were grade 2 and 30% were grade 1. There was a significant difference between before, after 3-months and 6-months follow-up (P=0.001*, P=0.001* respectively). Both the boxcar and the rolling scar types were significantly improved in both groups, yet the ice pick scar type didn't show significant improvement in any of the studied groups.

Regarding the quantitative score, in group A: before treatment; the score ranged from 13-27 with mean 19.20 ± 3.97. 6 months later; it ranged from 6-14 with mean 9.70 ± 3.37. There was a significant improvement in comparing scores before treatment, 3-months and 6-months follow-up (P= 0.019*, P=<0.001* respectively). In group B: before treatment; the score ranged from 12-36 with mean 19.80 ± 6.34. six months later; it ranged from 6-23 with mean 13.20 ± 5.39. There was a significant improvement in the mean values after 3-and 6-months of treatment in comparison to before treatment (P= 0.007*, <0.001* respectively). The blinded opinion of three observers showed excellent to very good improvement in 70% of patients without significant difference between the two groups. Also, both groups showed 60% patients' satisfaction with no significant difference (Figs. 2 & 3).

The histopathological examination showed increased epidermal thickness, decrease in perivascular inflammatory infiltrate, and more condensed collagen fibers in specimens from both groups (Figs. 4 & 5). Mallory trichrome stain showed more condensed, packed, organized, and parallel to the epidermis collagen bundles in both groups. Verhoeff-Van Gieson stain showed thicker, longer and more arranged elastic fibers in sections from both groups.

Electron microscopy of nanofat specimen showed complete destruction of fat cells, with loss of the normal architecture of fat tissue. There were remnants of fat cells with abnormal nucleus showing chromatin condensations with disrupted cell membrane. Some areas showed ghosts of cells with indistinguishable cytoplasmic organelles while some areas showed cells with multiple nuclei (euchromatic active dividing cells) which may be regenerative stromal cells (Figs. 6 &7).

No serious side effects were reported. Most patients experienced pain and oedema. 2 patients had post injection hyperpigmentation which faded gradually along 3 months after injection in group A and 1 patient had hypersensitivity reaction in the form of mild angioedema in the morning in the 1st month after treatment in group B.

4. DISCUSSION

Acne scarring is a visible indelible reminder of acne vulgaris with a wide range of scarring manifestations, from barely visible to severely disfiguring scars. It is psychological devasting condition and affects the quality of life of patients. Although it creates significant concerns for patients and physicians alike, there is currently no golden therapeutic option to treat it [14].

The separation of ADSCs and SVF cells in clinical applications is complicated, time consuming, costly and safety concerns exist [15]. [7] adopted that nanofat is devoid of viable adipocytes and retain ADSCs with their regenerative potentiality [7]. So, their study stimulated the use of nanofat graft in scar revision irrespective of its aetiology [16]. The idea of using fat for its regenerative properties not only for filling was first noticed by Coleman when he reported a case with acne scars that showed visual improvement of skin quality with subdermal lipofilling [11].

Table 1. Goodman's qualitative global acne scarring grading system [9,10]

Grades of post acne scars	Level of disease	Clinical features
1	Macular	These scars can be erythematous, hyper- or hypopigmented flat marks
2	Mild	Mild atrophic scars that may not be obvious at social distances of 50 cm or greater and may be covered adequately by makeup or the normal shadow of shaved beard hair in men
3	moderate	Moderate atrophic scars that is obvious at social distance of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in men, but still able to be flattened by manual stretching of the skin
4	Severe	Severe atrophic scarring that is evident at social distances greater than 50 cm and is not covered easily by makeup or the normal shadow of shaved beard hair in men and is not able to be flattened by manual stretching of the skin

Table 2. Demographic data

		Group A (n=20)	Group B (n=20)	p
Age (years)	Min. – Max. Mean ± SD.	22.0 – 49.0 30.1 ± 8.1	20.0 – 51.0 32.3 ± 8.8	P = 0.298
Duration (years)	Min. – Max. Mean ± SD.	4.0 – 30.0 11.80 ± 8.04	7.0 – 30.0 15.40 ± 6.85	P = 0.044
Sex	Male (n.) Female (n.)	12 (60%) 8 (40%)	8 (40%) 12 (60%)	P = 0.533
Skin type	Type III (n.) Type IV (n.)	12 (60%) 8 (40%)	10 (50%) 10 (50%)	P = 0.541
Acne scar type	Boxcar (n.) Rolling (n.) Ice pick (n.)	8 (40%) 14 (70%) 4 (20%)	10 (50%) 12 (60%) 4 (20%)	P= 1.000 P= 1.000 P= 0.669
Family history	Yes (n.)	8 (40%)	8 (40%)	P = 1.000

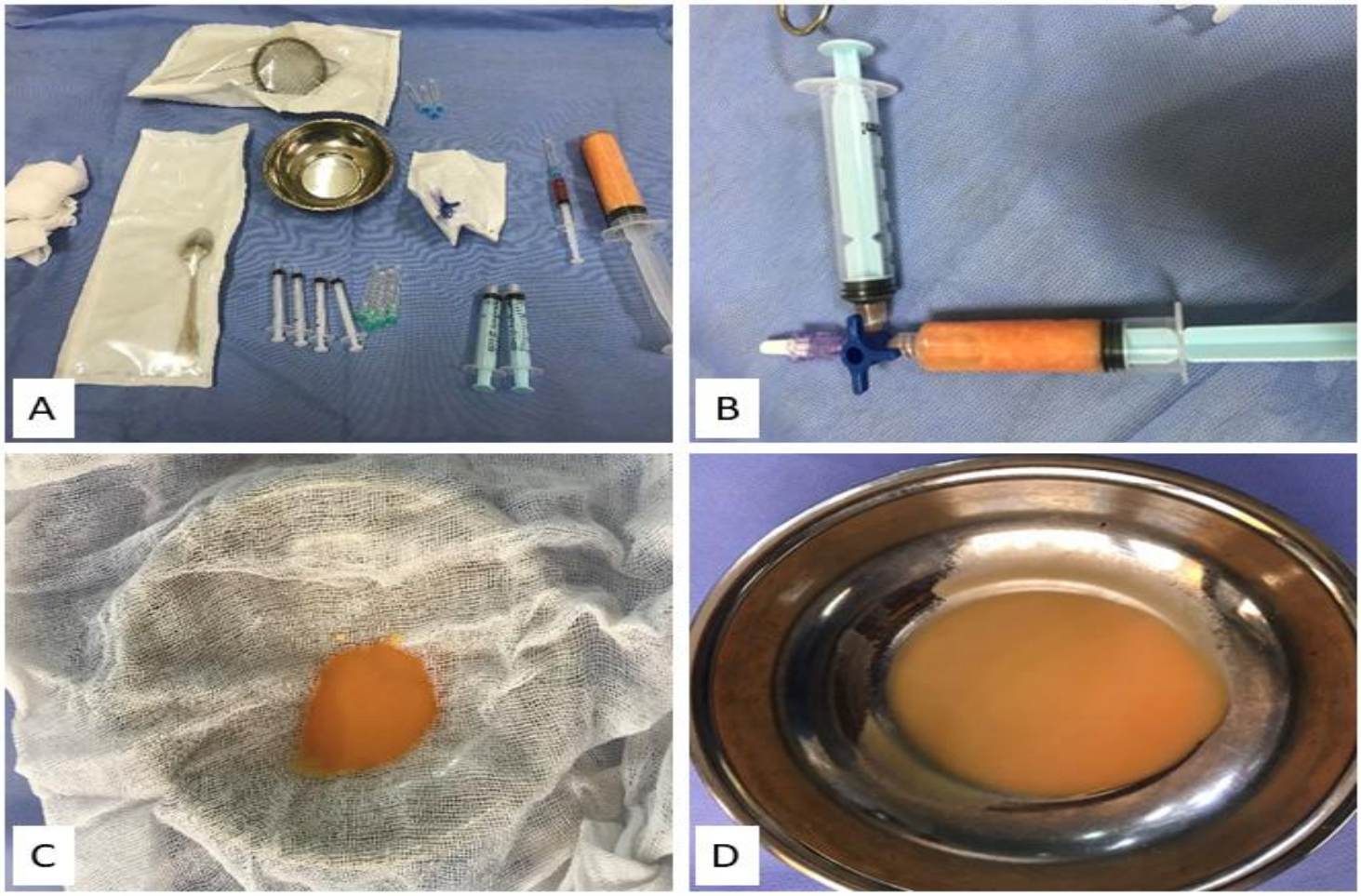

Fig. 1. Nanofat preparation

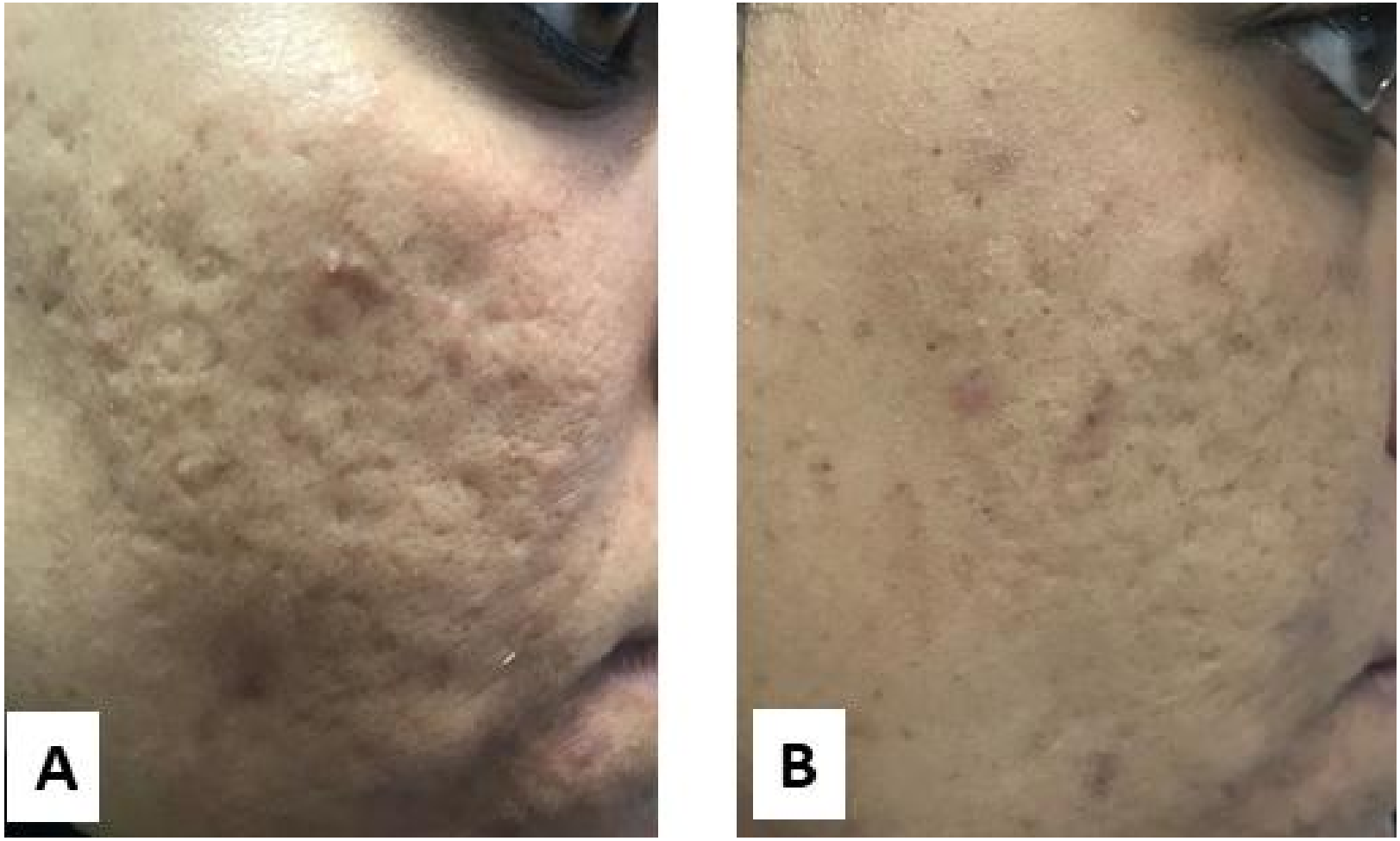

Fig. 2. 35-year-old female patient with excellent improvement in group A

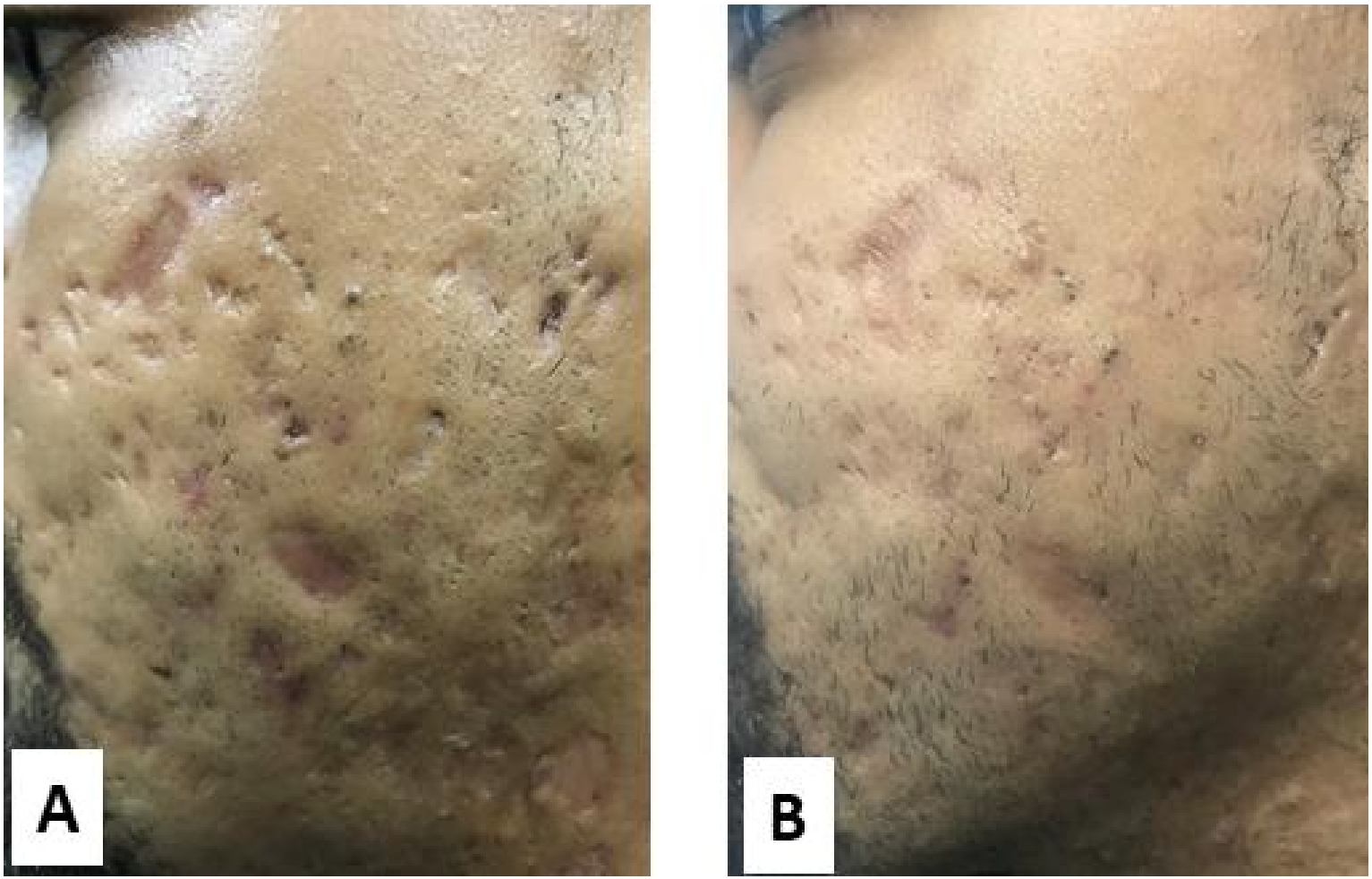

Fig. 3. 22-year-old male patient with excellent improvement in group B

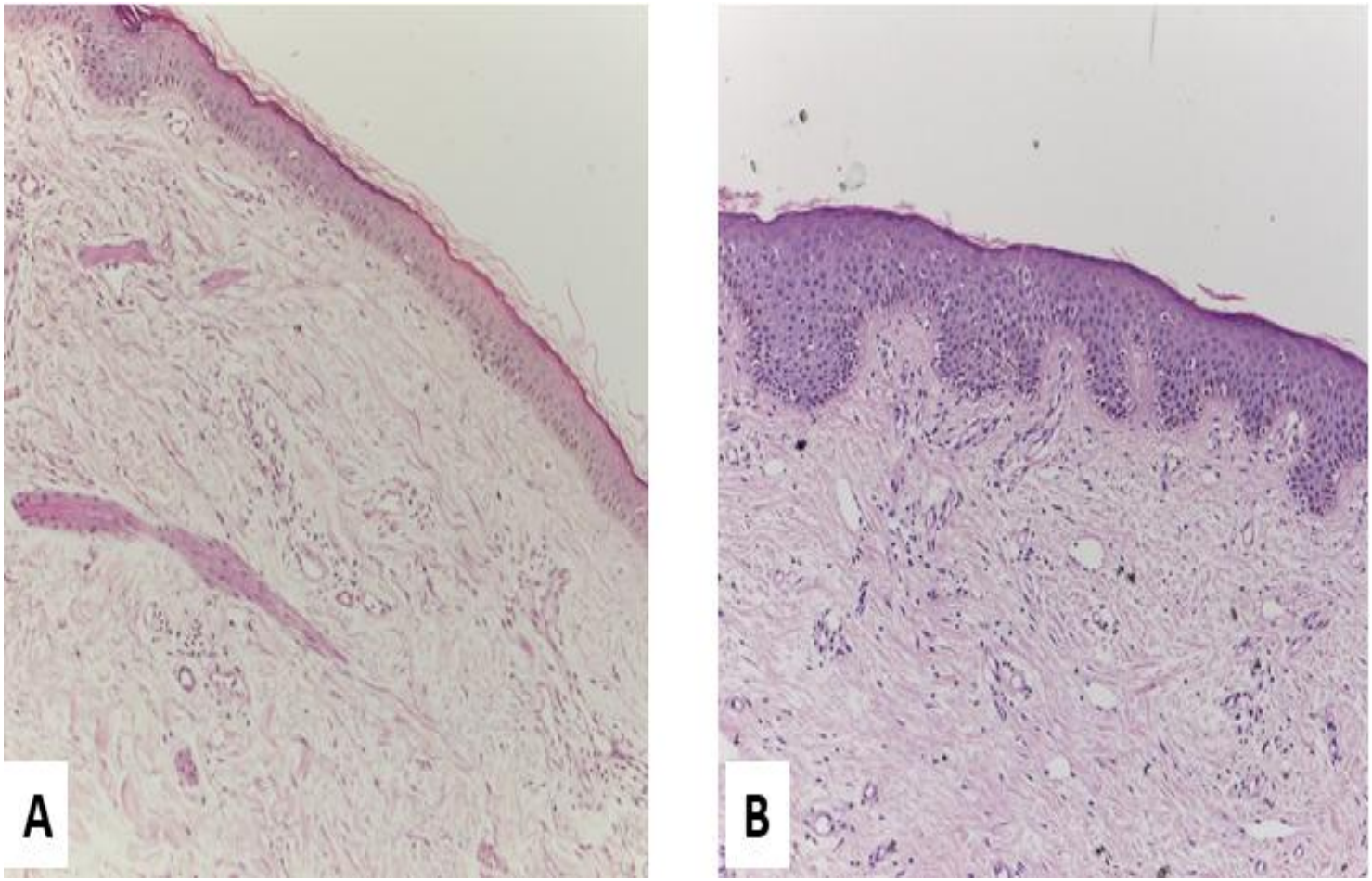

Fig. 4. H&E sections before (a) and 6-months after (b) treatment in group A showing increase in epidermal thickness, number, and length of rete ridges

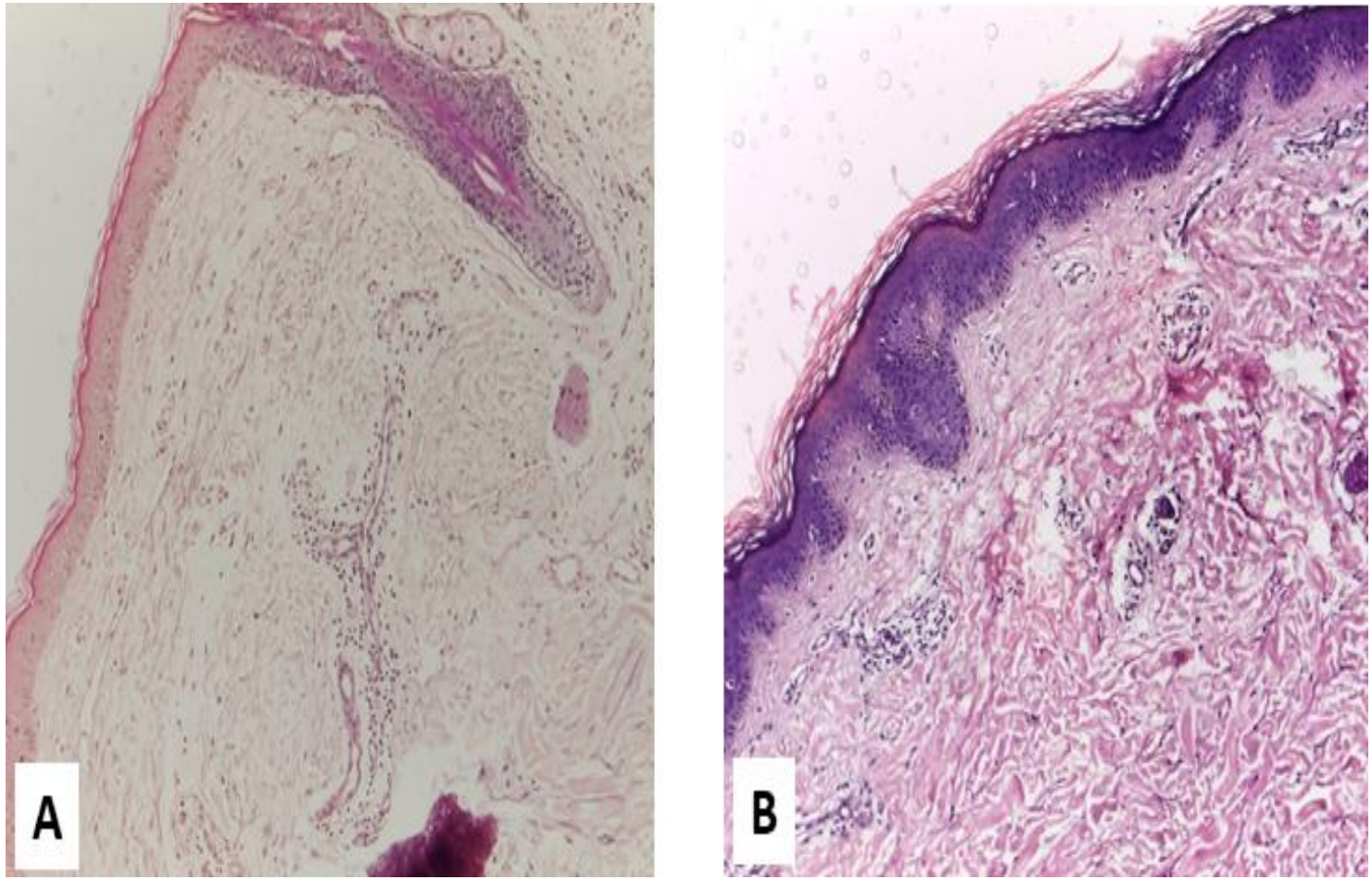

Fig. 5. H&E sections before (a) and 6-months after (b) treatment in group B showing increase in epidermal thickness, number, and length of rete ridges

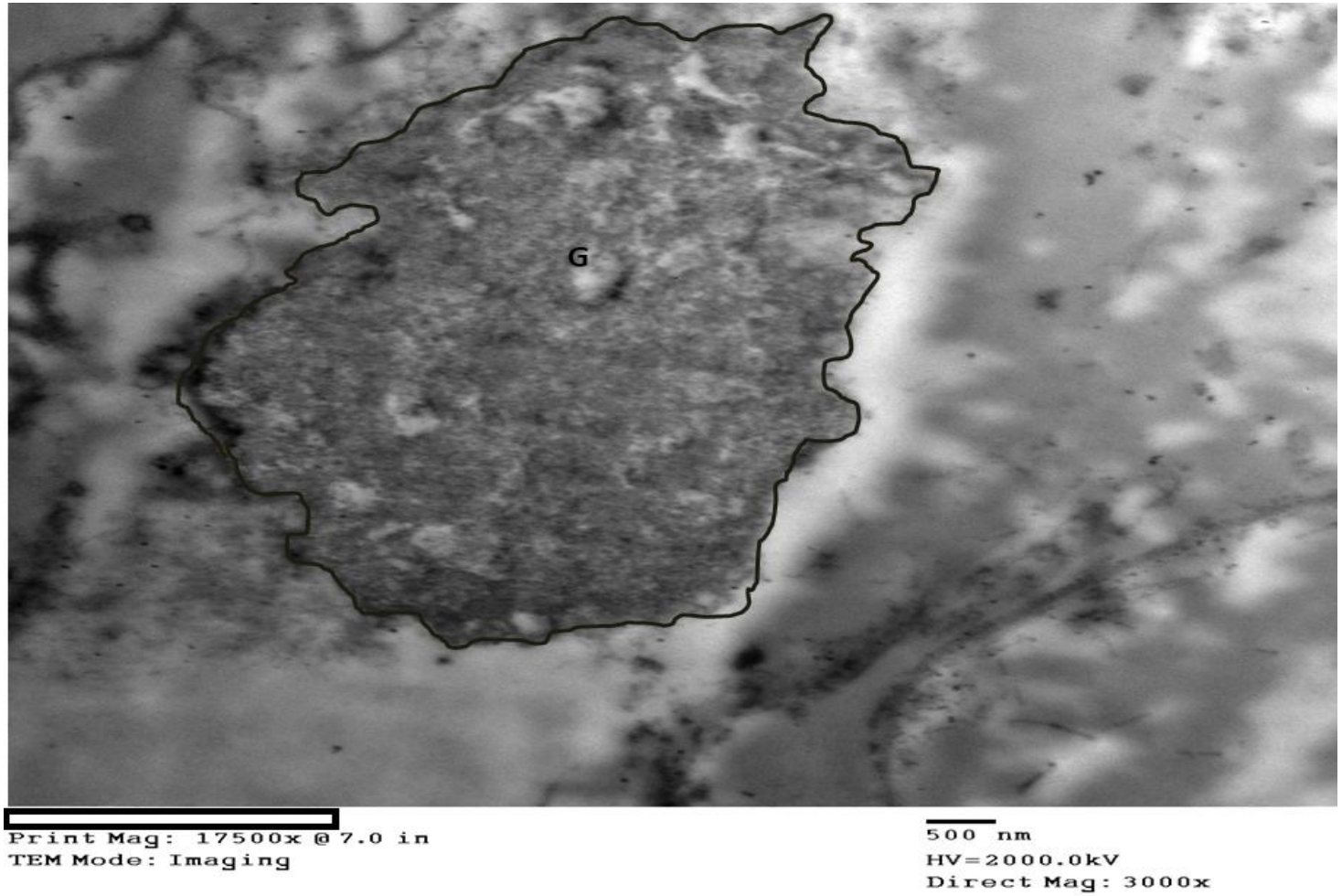

Fig. 6. Electromicrograph of nanofat specimen showing ghost cell with indistinguishable cytoplasmic organelles (G)

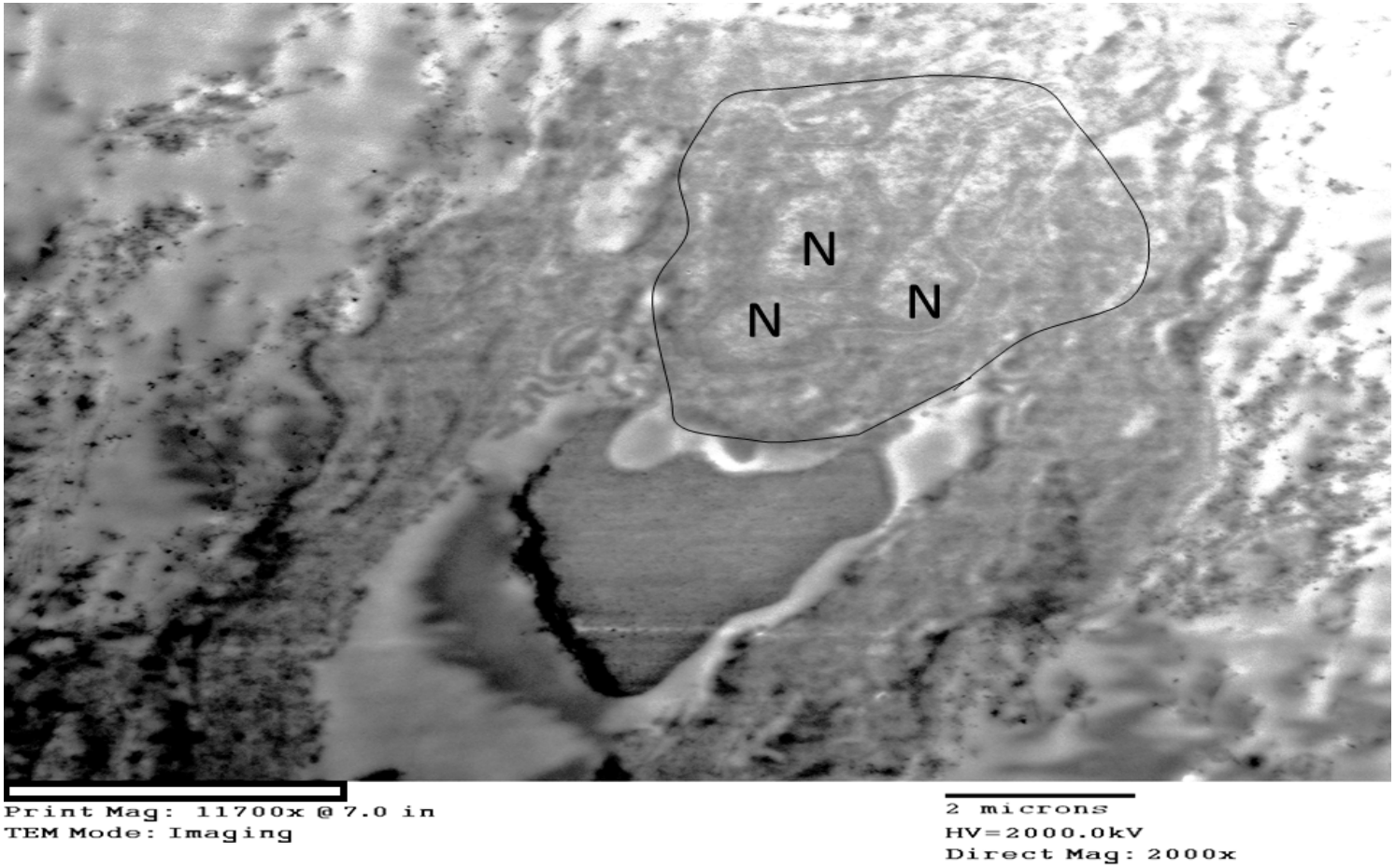

Fig. 7. Electromicrograph of nanofat specimen showing cell with multiple nuclei (euchromatic)

Rohrich et al. [17] studied the difference in adipocyte viability and quantity of ADSCs from different donor areas and adopted no significant differences between abdomen, thighs and flanks [17,18] found that lipoaspirate from lower abdomen is easily accessible and has a significant increase in ADSCs concentration than other sites [18]. In the current study, lipoaspirate was from lower abdomen in all cases.

We studied the effect of nanofat with or without enhancement on the atrophic acne scars. Patients treated with nanofat showed a statistically significant improvement in both qualitative and quantitative grading with patient satisfaction reached 60% based on improvement in the skin quality, decrease in the sebum secretion, decrease in the visibility and depth of individual scars. [19] used nanofat-PRP mixture and reported improvement in skin quality and appearance [19,16] treated 34 patients with intradermal nanofat injection and significant improvement in thickness, texture, irregularity of the scars was noticed[17]. Another study showed that scars became softer, less prominent with improvement of skin quality and appearance with 74% of patients rated good satisfaction [20].

It was demonstrated that mechanical procedure of shuffling lipoaspirated fat does not alter tissue viability and had no impact on SVF regenerative power [21]. ADSCs in nanofatwere found to constitute approximately 3 folds more over than that in standard lipoaspirate [22].

Patients in group B showed a statistically significant improvement regarding qualitative and quantitative grading with patient satisfaction reached 60%. The scars became less different compared to normal skin. To the best of our knowledge, no previous studies were conducted on enhanced nanofat with ADSCs. ADSCs are used as adjuvant to lipofilling as they augment the viability of fat graft. It was demonstrated that co-transplantation of ADSCs or SVF with fat would improve graft survival either through paracrine effects or direct differentiation into adipocytes [23-26] were the first to report that ADSCs could induce scar repair and fibroblast activation [24,25] injected ADSCs and showed significant improvement in scar appearance, increase in fibroblasts number at scar sites and enhanced collagen expression [25,26] found a significant decrease in grading scores before and after treatment by ADSCs injection [26].

In this study, although group B gave superior improvement over group A regarding qualitative score, patient satisfaction and physician's opinion, group A showed superior results than group B regarding quantitative score. However, there were no statistically significant difference between both groups. The insignificant difference was a question as it was expected that enhanced nanofat would give better results than nanofat alone. Li et al. [27] had an explanation for that as they found that fat grafts with larger ADSCs density have worse survival compared to that for grafts with an optimal density of 105 /ml ADSCs [27]. Similarly [28]. demonstrated that over supplementation of SVF may decrease fat graft survival by increasing metabolic load [28].

We used PRP as a carrier to the ADSCs pellet, as PRP has many GFs which may promote proliferation of ADSCs and potentiate their action [29]. Lei et al. [30] claimed that nanofat along with PRP had a better effect than the grafts that did not contain PRP [30]. It was found that nanofat PRP mixture resulted in higher weight, higher degree of angiogenesis, better survival and decrease in number of inflammatory cells [8,22]. The name of the emulsified fat differs between many authors either nano or microfat and its role does it related to fat cell or stemcells, enzymes and GFs contained so we studied the end emulsified fat by electron microscopy. It showed complete destruction of all adipocytes, with loss of the normal architecture of fat tissue. There were remnants of fat cells with abnormal nucleus showing chromatin condensations with disrupted cell membrane. Some areas showed ghosts of cells with indistinguishable cytoplasmic organelles while some areas showed cells with multiple nuclei which are euchromatic active dividing cells that may be regenerative stromal cells. Lei et al. [30] found in a nanofat sample examination by electron microscopy; many damaged fat cells with visible extracellular matrix and vascular components [30].

In group A, 2 patients experienced transient post-injection hyperpigmentation which faded gradually within 3 months. [31] observed that ADSCs may positively influence melanocytes migration to basal cell layer or they may differentiate into melanocytes [31]. In group B, one patient had a hypersensitivity reaction in the form of mild angioedema around eyes only in the morning in the 1st month after treatment. It may be due to one of chemical materials used in the isolation of ADSCs. A study adopted that residual collagenase is potentially toxic and associated with the risk of allergic reactions [32].

5. CONCLUSION

Both studied techniques; nanofat and ADSCs enhanced nanofat injections were safe and effective in treating atrophic acne scars without a significant difference. They did not only improve the scars but also, improved the skin texture and quality. However, Nanofat is cost-effective, easy, not time-consuming and its preparation does not need expensive chemicals compared with the ADSCs where their preparation takes hours, need extensive manipulation and usage of chemicals.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of this research article and accompanying images. Detailed informed consent before operation, including the nature of treatment, procedure, explanation of the operative steps, risks and expected results was signed.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The study protocol was confirmed by the ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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